XXXVIII.

Turkish Microbiology Congress

with International Participation

Starlight Hotel & Convention Center, Antalya

November 4 - 8, 2018

10th International Molecular and Diagnostic Microbiology Congress

Ankara Society of Microbiology

International Symposium on Migration, Travel & Infection

Turkish Society of Microbiology

Turkish Society for Parasitology

November 6th 2018

BRUCELLOSIS

RE-EMERGING ZOONOTIC AND FOOD BORNE DISEASE

Vaso Taleski

University „Goce Delchev”

Faculty of Medical Sciences, Shtip

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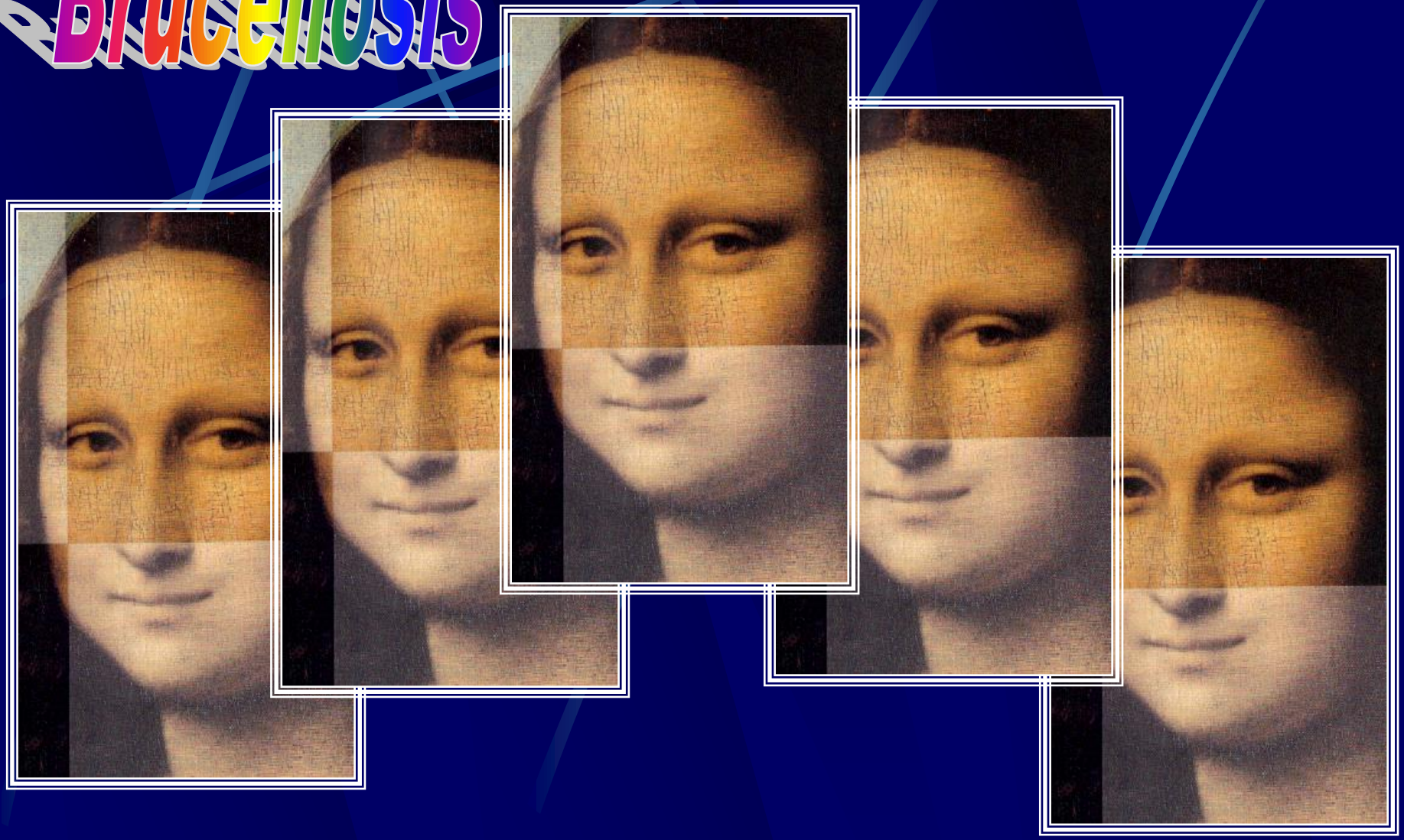
Brucellosis



ZOONOTIC DISEASES *(75% of infectious diseases)*

Disease	Main reservoirs	Usual mode of transmission to humans
Anthrax	livestock, wild animals, environment	direct contact, ingestion
Animal influenza	livestock, humans	may be reverse zoonosis
Avian influenza	poultry, ducks	direct contact
Bovine tuberculosis	cattle	milk
Brucellosis	cattle, goats, sheep, pigs	dairy products, milk
Cat scratch fever	cats	bite, scratch
Cysticercosis	cattle, pigs	meat
Cryptosporidiosis	cattle, sheep, pets	water, direct contact
Enzootic abortion	farm animals, sheep	direct contact, aerosol
Erysipeloid	pigs, fish, environment	direct contact
Fish tank granuloma	fish	direct contact, water

Erysipeloid	pigs, fish, environment	direct contact
Fish tank granuloma	fish	direct contact, water

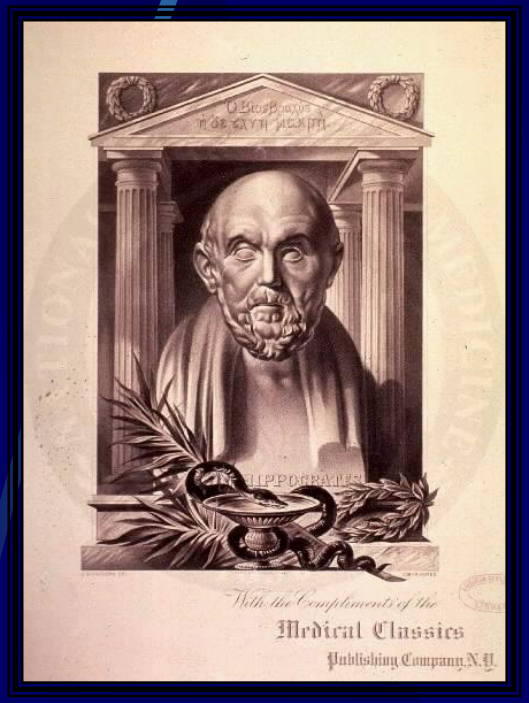
Food- and waterborne diseases and zoonoses Programme

The ECDC FWD Programme covers the following diseases:
anthrax, botulism, brucellosis, campylobacteriosis, cholera, cryptosporidiosis, echinococcosis, giardiasis, hepatitis A, legionellosis, leptospirosis, listeriosis, norovirus infection, salmonellosis, shigellosis, toxoplasmosis, trichinellosis, typhoid and paratyphoid fever, variant Creutzfeldt-Jakob disease, Shiga toxin/verocytotoxin -producing *Escherichia coli* (STEC/VTEC) infection, and yersiniosis.

The programme aims to support European Member States in the surveillance of food- and waterborne diseases and zoonoses and in responding to multi-country outbreaks.

Brucellosis

(Gastric Intermitent fever, Febris undulans, Malta fever, Mediterran fever, Neapolitan fever, Melitococcosis, Texas fever, Bang's disease, Febris melitensis)



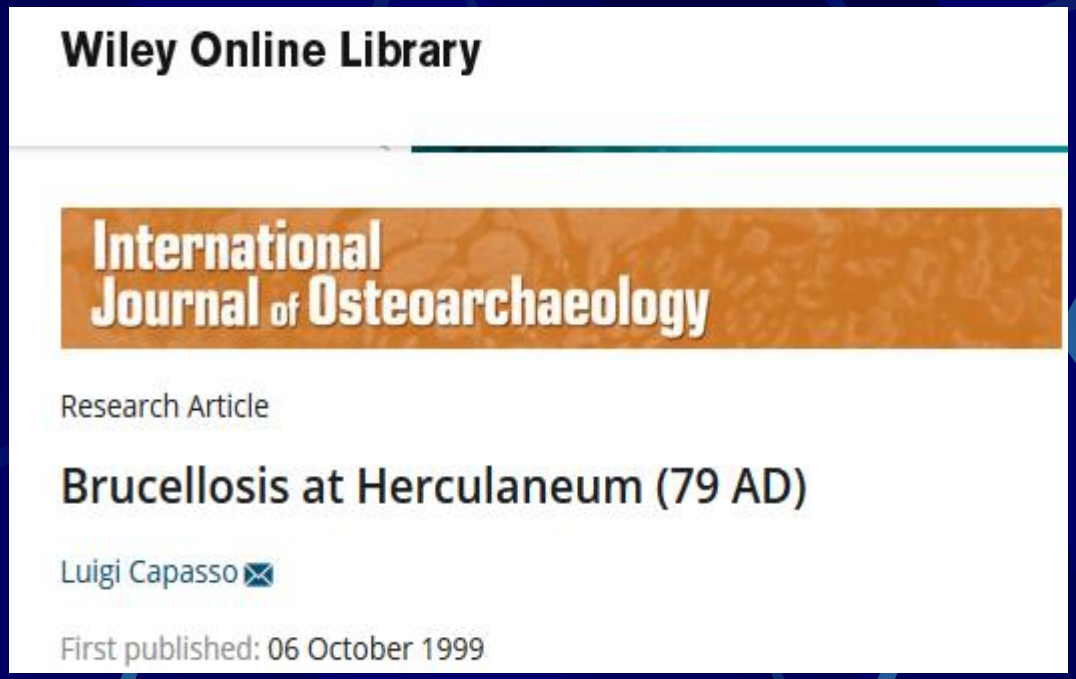
In the year 1853, Jeffery Allen **Marston** made the **first accurate description** of the disease in British army troops serving in Malta during the Crimean war

- **Hipocrates (450 BC)**

What happened in Pompeii
and
Herculaneum

August 24, 79 before Christ ?


3D.PAT



The author describes **vertebral lesions** observed in the skeletons of 16 adults who fled to the ancient beach at **Herculaneum** during the eruption of Mount Vesuvius, where they were buried alive.

Written sources mention that the **Romans made considerable use of milk from sheep and goats**. It was **consumed directly, without cooking or other forms of treatment.**

J Infect. 2002 Aug;45(2):122-7.

Bacteria in two-millennia-old cheese, and related epizoonoses in Roman populations.

Capasso L[1].

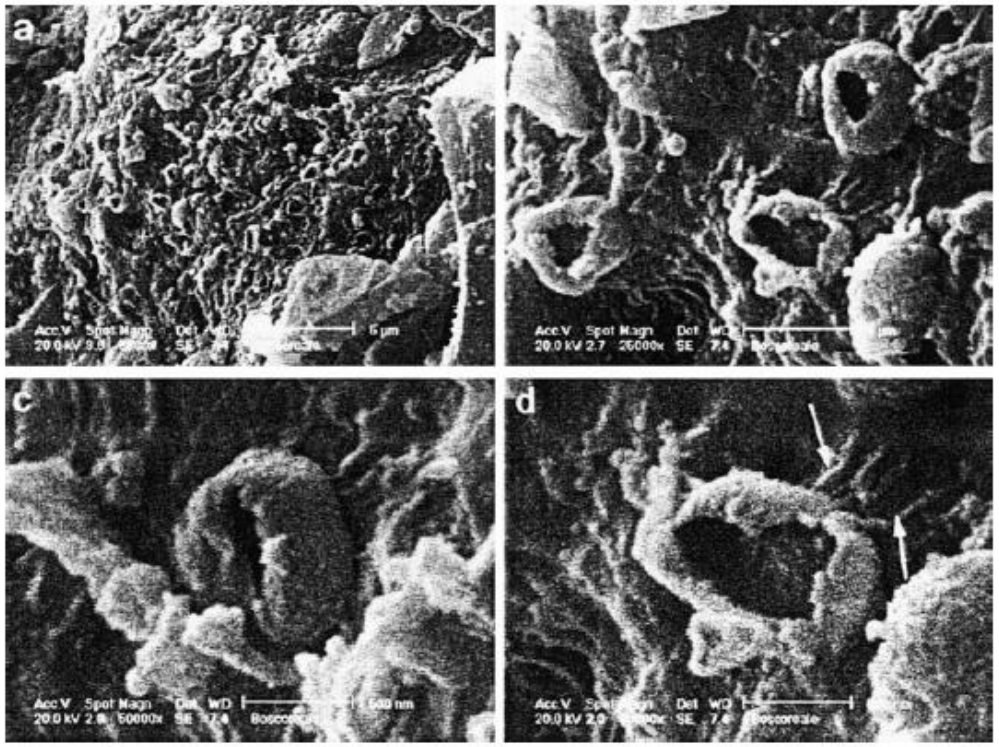


Figure 5. Cocci-like bacterial particles in a high-density colony (a: 5000×) in the Herculaneum carbonized cheese (79 AD); The monomorphic and monodimensional cocci (around 0.8 μ) show large holes with invaginated borders (b: 25,000×). In some case we can estimate the thickness of bacterial-wall (about 10 mμ) (c: 50 000×), and we can demonstrate the presence of proteinic bridges between the bacterial walls and the cheese mixture (arrows in d: 50,000×). These remain of bacterial walls are morphologically comparable with *Streptococci* or *Brucellae*.

Brucellosis

Incubation : 1 week to 2-3 months (appr. 3weeks)

Spectrum of clinical manifestations:

- „Undulant fever", night sweats, chills, malaise, often accompanied by severe headache, myalgias, arthralgias.
- Lymphadenopathy, splenomegaly, hepatomegaly

Complications:

- Meningoencephalitis, cerebellar abscess,
- Granulomatous hepatitis, hepatic and splenic abscesses, cholecystitis,
- arthritis, spondylitis, osteomyelitis
- Endocarditis,
- Granulomas in kidneys, orchiepididimitis etc.

Humans become infected by:

1. Ingestion of unpasteurized - contaminated milk and dairy products or by (80% Nicoletti, 2009)

2. Direct or indirect contact with animals or with contaminated animal products (10% Nicoletti, 2009)

3. Inhalation of aerosols.

- ***Sir David Bruce* (1887 - Malta)**

Brucella melitensis; **in spleen of death soldier**

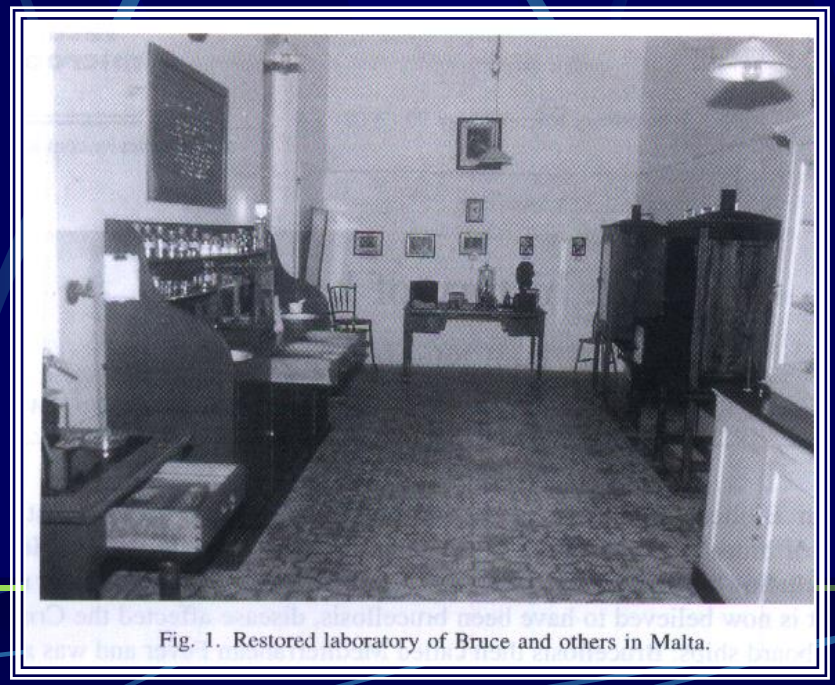


Fig. 1. Restored laboratory of Bruce and others in Malta.

> *B. abortus,* 1897, Copenhagen, *Bang*
in cows with abortions

>*B. suis,* 1914, *Traum* from Swine fetus

>*B. ovis* 1953, *Buddle & Boyes* at New Zeland
from ships with genital diseases

>*B. neotomae,* 1957, Juta Utah USA, *Stoenner&Lackman,*
in woods rats

> *B. canis,* 1968, *Carmichael & Bruner, USA*
dogs with abortions.

Brucella species:

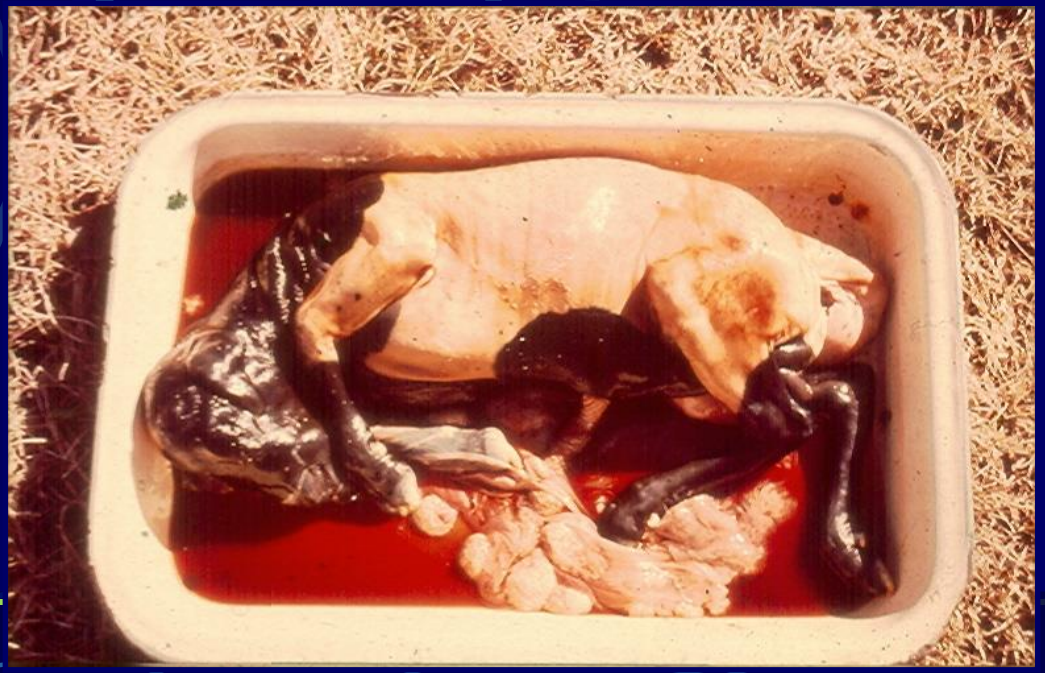
1. B. melitensis*	**16M**	**biotype 1**
	63 / 9	**biotype 2***
	Ether	**biotype 3**

2. B. abortus*	**544**	**biotype 1**
	86 / 8 / 59	**biotype 2**
	Tulya	**biotype 3**
	292	**biotype 4**
	B3196	**biotype 5**
	870	**biotype 6**
	63 / 75	**biotype 7**
	C68	**biotype 9**

3. B. suis*	**1330**	**biotype 1**
	Thomsen	**biotype 2**
	686	**biotype 3**
	40	**biotype 4**

4. B. canis*	**RM6 / 66**	

5. B. ovis	**63 / 290**	
6. B.neotomae	**5K33**	



Since 1994, isolated **novel *brucella* species**:

- ***B. ceti*** (marine mammals/ **whales, dolphins, porpoises**

- ***B. Pinnipedialis*** **(seals and sea lions)**

“Atypical *brucella*”

- ***B. microti*** (voles in Czech Republic, red foxes in Austria, Hungarian wild boar)

- ***B. Inopinata*** (2010, isolated from breast implant and blood of woman of age of 71, host unknown)

New "Atypical *brucella*"

- B. papionis

- B. vulpis

Whatmore, A. M. *et al.* *Brucella papionis* sp. nov., isolated from baboons (*Papio* spp.). *Int. J. Syst. Evol. Microbiol.* **64**, 4120–4128 (2014).

Brucella evolution & taxonomy

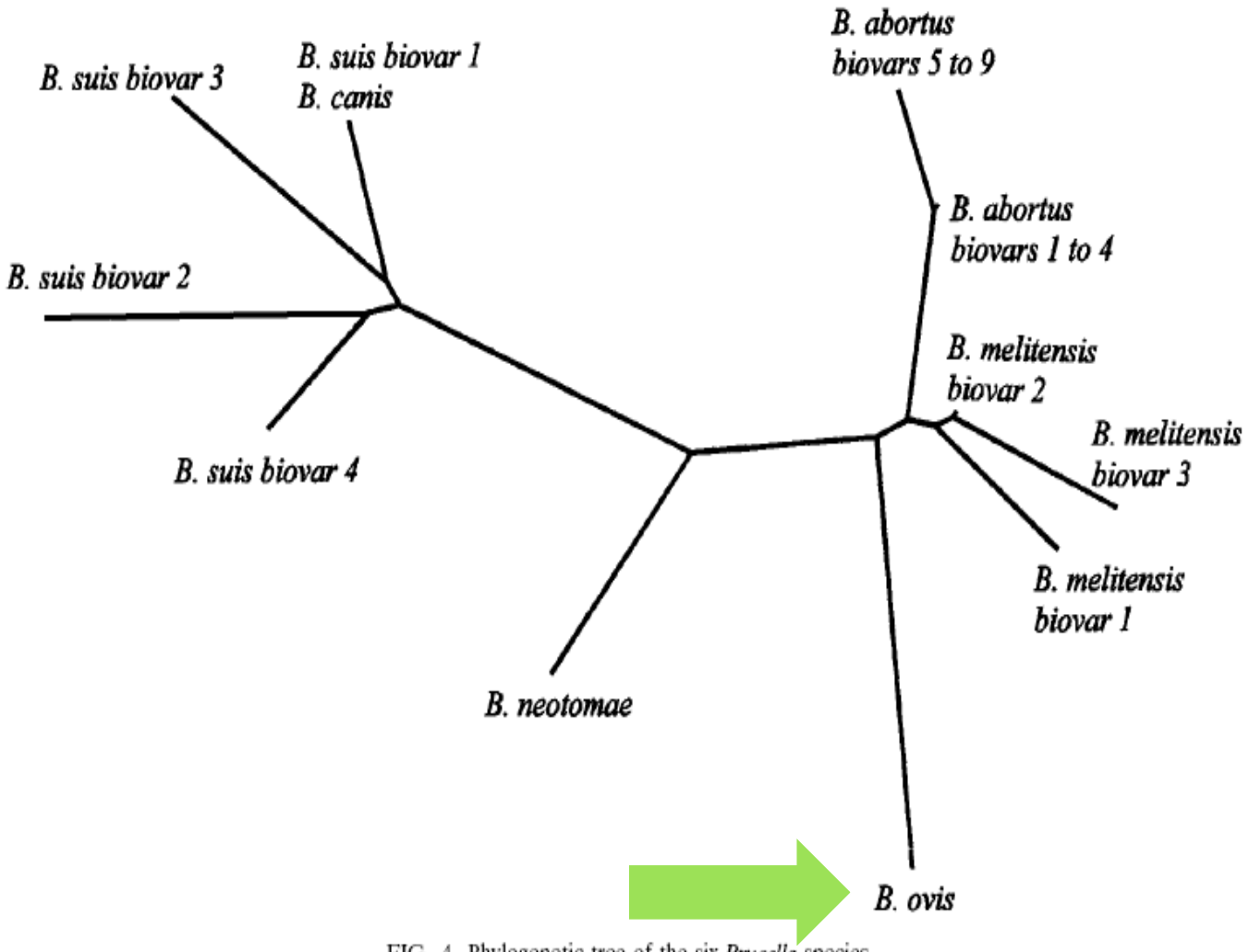
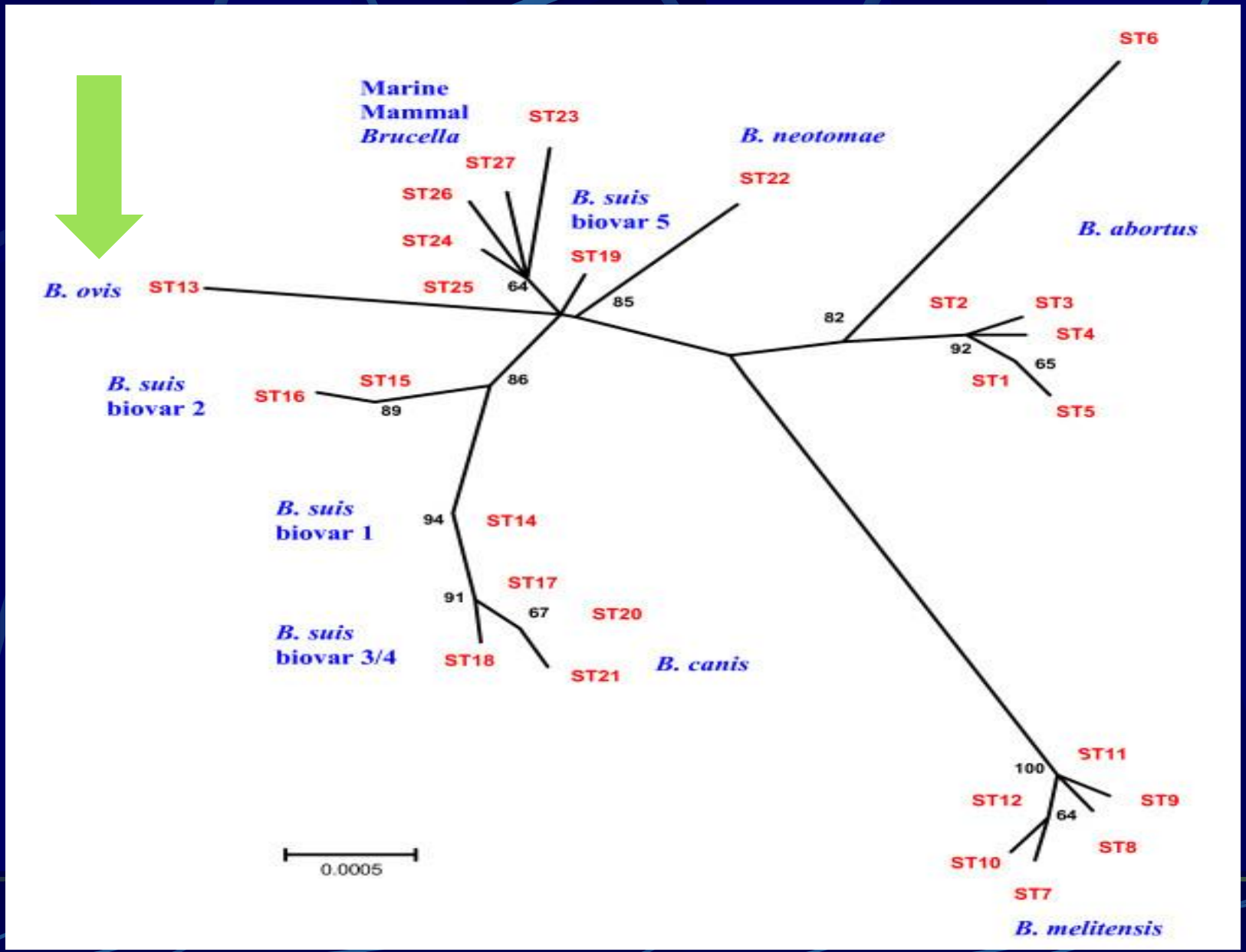
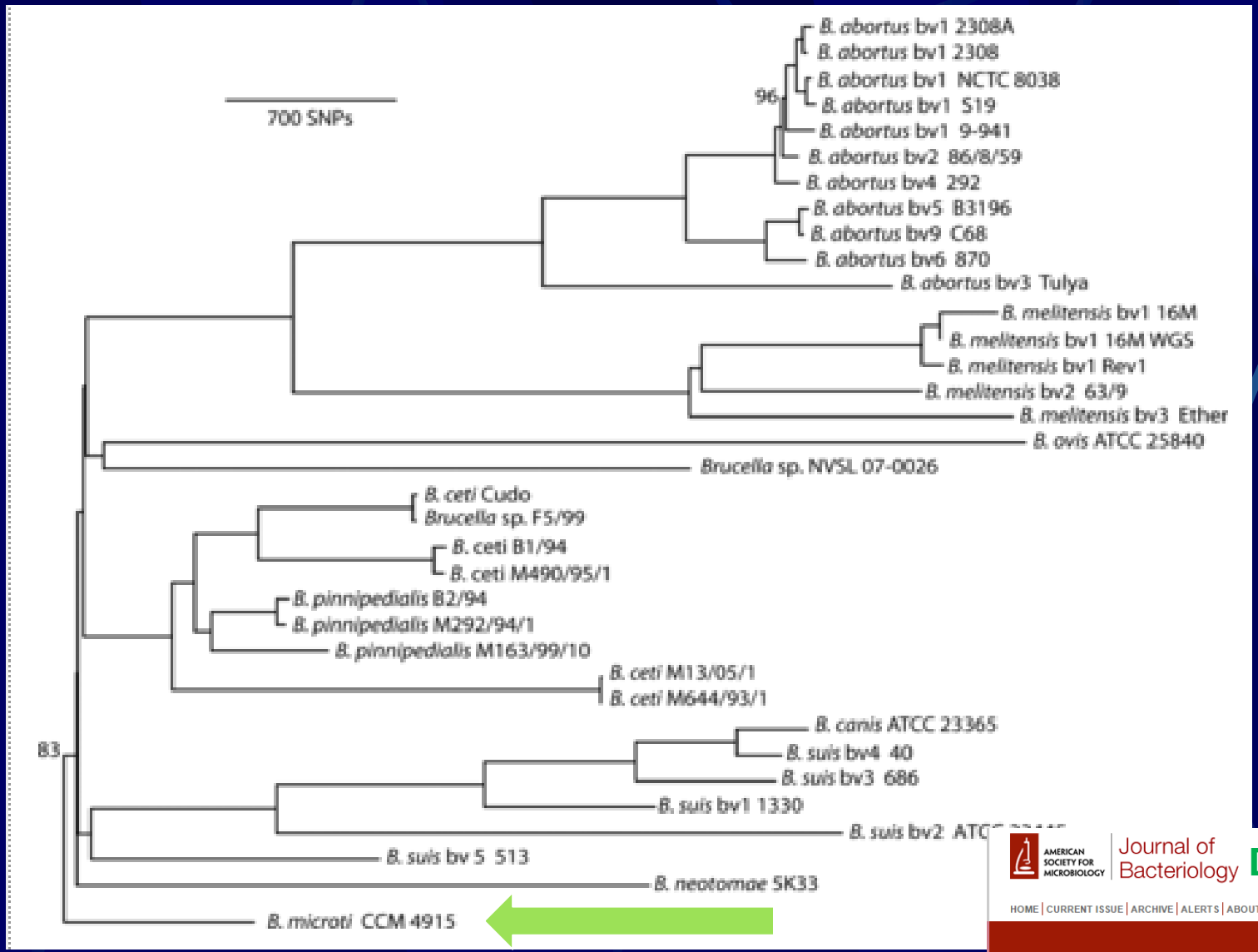


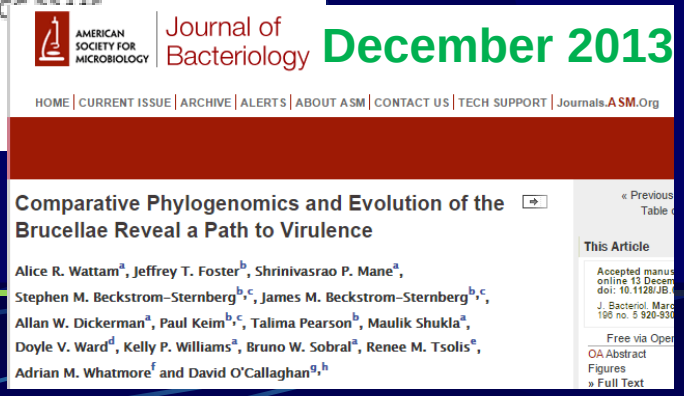
FIG. 4. Phylogenetic tree of the six *Brucella* species.

Brucella evolution & taxonomy





Phylogenetic analysis, showing a rapid radiation following the divergence of ***B. Microti***



Taxonomy of brucella is still controversial and debating is still going on.

It has been suggested that divergence of species *brucella* was a result of divergence of their hosts, 60 million years ago.

But, divergence of hosts of *B. ceti* and *B. pinnipedialis* happened:

- **before about 35 million years for seals and sea lions**
- **before 55 million years for whales and dolphins**

Biggest number of known, classical *brucella* had divergence from their common ancestor *B. ovis, probably about 86.000 to 296.000 years ago.*

=> Divergence of *brucella* of marine mammals is incompatible with divergence of their hosts.

Following the evolution of *B. microti,* first evolution was divergence to two following speciae: *B. suis* and *B. neotomae* (Audic, 2011).

QUESTIONS:

1. Infection of marine mammals happened through food chain from terrestrial mammals?

2. Marine mammals *brucella* infected terrestrial hosts?

Vet Res. 2005 May-Jun;36(3):313-26.

From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis.

Godfroid J, Cloeckaert A, Liautard JP, Kohler S, Fretin D, Walravens K, Garin-Bastuji B, Letesson JJ.

Lancet Infect Dis. 2006 Feb;6(2):91-9.

The new global map of human brucellosis.
Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV.

Int J Antimicrob Agents. 2010 Nov;36 Suppl 1:S8-11. doi: 10.1016/j.ijantimicag.2010.06.013. Epub 2010 Aug 8.

The changing Brucella ecology: novel reservoirs, new threats.

Pappas G.

The new global map of human Brucellosis

Several areas traditionally considered to be endemic (e.g., France, Israel (?), and most of Latin America have achieved **control** of the disease.

On the other hand, **new foci** of human brucellosis have emerged, particularly in central Asia, while the situation in certain countries of the Near East (e.g., Syria) is rapidly worsening.

The disease is still present, in varying trends, both in European countries and in the USA.

Brucellosis up 83% in Israel, called a 'Third world epidemic'

Posted by Robert Herriman on August 2, 2015 // 1 Comment

The number of human cases of the zoonotic infection, brucellosis, has increased in parts of Israel by 83 percent, prompting one physician to call it a "Third world epidemic".

The Jerusalem Post reports the surge in cases in centered around Beduin inthe South and other Arabs in eastern Jerusalem, Nazareth, Acre and elsewhere in the North.

MK Ahmad Tibi, a physician said, "Just in the last six months, 217 cases were reported. There is no excuse for this negligence, because Israel has a very high level of medical and agricultural know how."

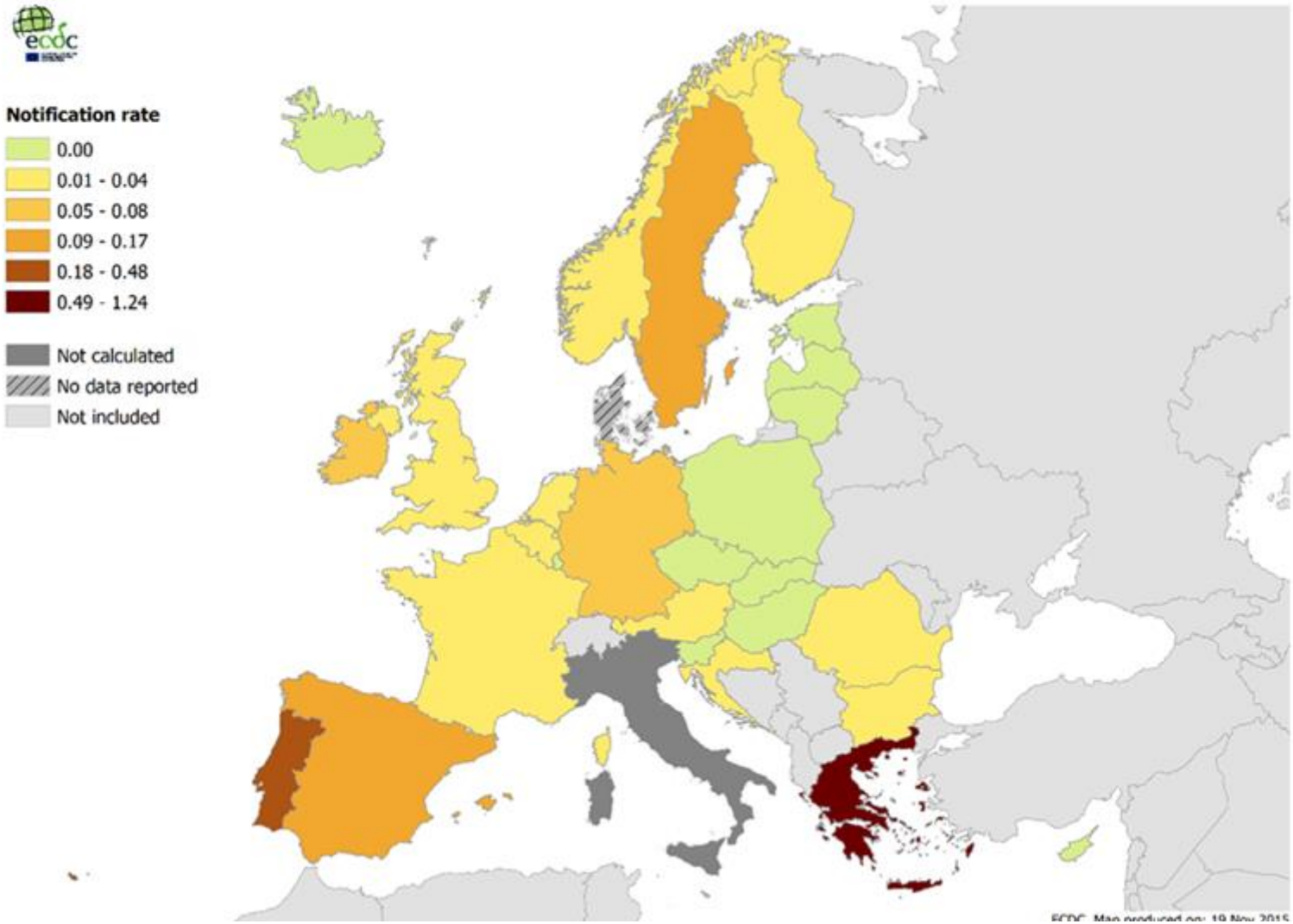
In addition, hospitalizations for the infection are up 30 percent compared to last year.

Health officials say that experts have been sent to teach the public about how to make milk safe; however, they note, "but they don't cooperate and listen, and they even hide the products from us, even though we have made it clear that they are causing themselves to get sick."

Brucellosis is one of the most serious diseases of livestock, considering the damage done by the infection in animals. Decreased milk production,weight loss, loss of young, infertility, and lameness are some of the affects on animals.

The Brucella species are named for their primary hosts: Brucella melitensis is found mostly is goats, sheep and camels, B. abortus is a pathogen of cattle, B. suis is found primarily in swine and B. canis is found in dogs.

Figure 1. Reported confirmed brucellosis cases: rate per 100 000 population, EU/EEA, 2014



Source: Country reports from Austria, Belgium, Bulgaria, Croatia, Cyprus, the Czech Republic, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Norway, Poland, Portugal, Romani Slovakia, Slovenia, Spain, Sweden, the United Kingdom.

Suggested citation: European Centre for Disease Prevention and Control. Annual epidemiological report 2015. Brucellosis. Stockholm: ECDC; 2016.

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Table 1. Reported confirmed brucellosis cases: numbers and rate per 100 000 population, EU/EEA, 2010–2014

Country	2010		2011		2012		2013		2014					
	Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate	National data	Report type	Reported cases	Confirmed cases	Rate	ASR
Austria	3	0	5	0,1	2	0	7	0,1	Y	C	1	1	0	0
Belgium	0	0	5	0	4	0	0	0	Y	C	1	1	0	-
Bulgaria	2	0	2	0	1	0	0	0	Y	A	2	2	0	0
Croatia	.	.	.	.	0	0	0	0	Y	C	1	1	0	0
Cyprus	0	0	0	0	0	0	0	0	Y	C	0	0	0	0
Czech Republic	1	0	0	0	0	0	0	0	Y	C	0	0	0	0
Denmark	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Estonia	0	0	0	0	0	0	0	0	Y	C	0	0	0	0
Finland	0	0	0	0	1	0	0	0	Y	C	1	1	0	0
France	20	0	21	0	28	0	19	0	Y	C	16	14	0	0
Germany	22	0	24	0	28	0	26	0	Y	C	47	45	0,1	0,1
Greece	97	0,9	98	0,9	123	1,1	159	1,4	Y	C	135	135	1,2	1,2
Hungary	0	0	0	0	0	0	0	0	Y	C	0	0	0	0
Iceland	0	0	0	0	0	0	0	0	Y	C	0	0	0	0
Ireland	1	0	1	0	2	0	1	0	Y	C	3	3	0,1	0,1
Italy*	171	0,3	166	0,3	184	0,3	137	0,2	N	C	8	8	-	-
Latvia	0	0	0	0	0	0	1	0	Y	C	0	0	0	0
Liechtenstein	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Lithuania	0	0	0	0	0	0	2	0,1	Y	C	0	0	0	0
Luxembourg	1	0,2	1	0,2	0	0	0	0	Y	C	0	0	0	0
Malta	0	0	0	0	0	0	1	0,2	Y	C	0	0	0	0
Netherlands	6	0	1	0	3	0	5	0	Y	C	1	1	0	0
Norway	2	0	2	0	4	0,1	2	0	Y	C	2	2	0	0
Poland	0	0	0	0	0	0	1	0	Y	C	1	1	0	0
Portugal	88	0,8	76	0,7	37	0,4	22	0,2	Y	C	54	50	0,5	0,5
Romania	2	0	1	0	0	0	0	0	Y	C	2	2	0	0
Slovakia	1	0	0	0	1	0	1	0	Y	C	0	0	0	0
Slovenia	0	0	1	0	0	0	0	0	Y	C	0	0	0	0
Spain	78	0,2	43	0,1	62	0,1	87	0,2	Y	C	70	60	0,1	0,1
Sweden	12	0,1	11	0,1	13	0,1	10	0,1	Y	C	16	16	0,2	0,2
United Kingdom	12	0	25	0	14	0	15	0	Y	C	11	11	0	0
EU/EEA	**519**	**0,1**	**483**	**0,1**	**507**	**0,1**	**496**	**0,1**	.	**C**	**372**	**354**	**0,1**	**0,1**

Source: Country reports. Legend: Y = yes, N = no, C = case based, A = aggregated, · = no data reported, ASR: age-standardised rate, - = no report

** Provisional data for 2014. Notification rates not calculated.*

A new outbreak of brucellosis in Bulgaria detected in July 2015 – preliminary report

R Nenova [1], I Tomova [1], R Saparevska [2], T Kantardjiev [1]

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2. Surveillance of Communicable Diseases Department, Regional Health Inspectorate – Kyustendil, Bulgaria

Correspondence: Rumiana Nenova (rnenova62@gmail.com)

Citation style for this article:
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Article submitted on 11 September 2015 / accepted on 01 October 2015 / published on 01 October 2015

During July 2015 a brucellosis outbreak was detected in Kyustendil district, west Bulgaria. As of 15 August, 31 patients have been diagnosed all with an epidemiological connection to Rila town. Patients have not travelled/worked abroad. Breeding family-owned goats and/or improper use of their milk appear to be the main risk factors for transmission of the infection. This second autochthonous brucellosis outbreak in Bulgaria since 2006, affects the western part of the country.

At the beginning of July 2015, a man in his late 30s presented at the National Reference Laboratory for High Medical Risk Infections (NRL HMRI) in Bulgaria for a consultation and testing in connection with undulant fever up to 38–38.5 °C, that had lasted for ca 30 days.

Outbreak in Kyustendil district

Within 24 hours of the diagnosis of the patient, an urgent notification was sent to the Regional Health Inspectorate (RHI), Kyustendil, and the Ministry of Health in accordance with Order 21/2005 for mandatory reportable infectious diseases [1]. Nineteen days after, two other patients, both goat-breeders from the same town as the first patient, were diagnosed with brucellosis. An epidemiological investigation was started immediately in Rila town (2,762 inhabitants). An alert was also sent to the Veterinary Health Service and testing of herds in the area was initiated.

As of 15 August, 31 human infections have been serologically confirmed at the NRL HMRI (Figure 2). Three of the infected persons have professions exposing

Psychiatria Danubina, 2014; Vol. 26, Suppl. 3, pp 546-551
Original paper
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MELITOCOCCOSIS IN THE REPUBLIC OF CROATIA

Željko Cvetnić[1], Silvio Špičić[1], Tomislav Kiš[2], Maja Zdelar-Tuk[1], Sanja Duvnjak[1], Ivana Račić[1], Miroslav Benić[1], Boris Habrun[1], Irena Reil[1] & Zvonimir Šostar[3]

[1]Croatian Veterinary Institute Zagreb (Department of Bacteriology and Parasitology), Zagreb, Croatia
[2]Ministry of Agriculture - Directorate for Veterinary Medicine and Food Safety, Zagreb, Croatia
[3]Andrija Stampar Teaching Institute of Public Health, Zagreb, Croatia

SUMMARY

Background: *Melitococcosis is one of the most widespread zoonoses worldwide. In the period from 2009 to 2013, comprehensive melitococcosis testing was conducted in the Republic of Croatia.*

Methods and results: *During the testing, the Rose Bengal test was applied to 344019 blood samples of sheep and goats, and positive reactions were confirmed in 1143 (0.3%) of samples. The complement fixation test (confirmatory test) was conducted on 43428 samples, with positive reactions confirmed in 768 (1.8%) of samples. The organs and tissues of 336 sheep and goats were inspected bacteriologically, and Brucella sp. was isolated in 15 (4.5%) of samples. Positive serological and bacteriological reactions were confirmed in the Karlovac, Lika-Senj and Split-Dalmatia Counties. Bacteriological and molecular techniques (Bru-up/Bru-low and Bruce-Ladder) in isolates proved the presence of Brucella melitensis biovar 3.*

Conclusion: *On the basis of this study, it can be concluded that Croatia has a favourable situation concerning the infection of ruminants with B. melitensis, and that ongoing controls of the disease are necessary.*

Key words: *melitococcosis - Brucella melitensis – ruminants - serological and bacteriological tests - Republic of Croatia*

* * * * *

INTRODUCTION

Melitococcosis or brucellosis is a chronic infectious disease of sheep and goats caused by the species Brucella (B.) melitensis. It is one of the most widespread zoonoses worldwide. Its appearance has a great influence on human and animal health, economic development and the agriculture and tourism of that country. It is particularly widespread in sheep and goats in the is present, and various brucellosis eradication programmes are ongoing (Pappas et al. 2006, Godfroid & Kasbahrer 2002, Taleski et al. 2002, Pappas 2010).

This paper gives an overview of the distribution of melitococcosis in the Republic of Croatia in the period from 2009 to 2013. Bacteriological and molecular techniques were used to prove and confirm the species Brucella sp.

International Atomic Energy Agency

IAEA Regional Technical Co-operation Project RER/5/012 “Regional Control of Brucellosis in Sheep and Goats”, Skopje-Ohrid-Macedonia, 14-18 April 2003

COST Action 845
Brucellosis in Animals and Man

INTERNATIONAL RESEARCH CONFERENCE FOR BRUCELLOSIS IN SMALL RUMINANTS

28.11. – 30.11.2005
Skopje, MACEDONIA

Brucellosis in SEE - Mediterranean Region
Struga, MACEDONIA, 12-14 November, 2009

BRUCELOZA U HRVATSKOJ I SUSJEDNIM ZEMLJAMA

HRVATSKA AKADEMIJA ZNANOSTI I UMJETNOSTI

Razred za medicinske znanosti

Odbor za animalnu i komparativnu patologiju

i

HRVATSKI VETERINARSKI

INSTITUT ZAGREB

organizirali su u srijedu, 25.9.2013. u palači Hrvatske akademije znanosti i umjetnosti, održavanje simpozija

BRUCELOZA U HRVATSKOJ I SUSJEDNIM ZEMLJAMA

Naučno-stručni simpozijum "Bruceloza u Jugoistočnoj Evropi"

Niš, Republika Srbija
16-19 Oktobar 2013

Milanski edikt, 313-2013, Srbija

Grad Niš

Veterinarska komora Srbije

Medicinski fakultet u Nišu

- **Serbia** /1980 to 2008 reported 1521 (Cekanjac at all., 2010).
- **Bosna & Hercegovina** 1980 to 2008 reported 1639 cases (Obradovic, 2010).
- **Croatia until** 1990 free from brucellosis, when epizooty between sheeps and goats appear in Istria. Proved reservoir of *B. suis* in wild boars (Taleski at all. / Cvetnic, Katalinic, 2002).
- **Bulgaria report on only two cases of human brucellosis for the period** 1996 to 2001 (Taleski at all. / Kantardziev, 2002), 37 cases in 2005 and 58 in 2007 (Nenova at all., 2013).
- **Turkey in** 2005, annual incidence of human brucellosis was 26,20 on 100.000 inhabitants.

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J Infect Dev Ctries. 2014 May 14;8(5):581-8. doi: 10.3855/jidc.3510.

Human brucellosis in Turkey: different clinical presentations.

Guler S[1], Kokoglu OF, Ucmak H, Gul M, Ozden S, Ozkan F.

Author information

Abstract

INTRODUCTION: Brucellosis is still endemic in Turkey and presents a major public health risk. The aim of this study wasto investigate the clinical and laboratory properties and complications of brucellosis cases.

METHODOLOGY: The files of 370 patients (162 males, 208 females) with brucellosis between March 2006 and January 2012 were analyzed retrospectively.

RESULTS: The mean age of patients was 39, 6±18.2 years. The major risk factor was unpasteurized dairy products in 155 (41.8%) cases. The complications included hematological (58.1%), osteoarticular (48.3%), hepatobiliary (26.7%),

VI Конгрес на микробиолозите на Македонија со меѓународно учество
VI Congress of Macedonian Microbiologists with international participation

FEMS-supported Symposium: "Emerging infections"

30.5 - 2.6.2018 год., Охрид, Р. Македонија

30.05 – 2.06.2018, Ohrid, R. Macedonia

Zoonoses

Zoo-1

INVESTIGATION OF CROSS-TRANSMISSION DYNAMICS OF BRUCELLOSIS OBSERVED IN CHILDREN IN THE PROVINCE OF DIYARBAKIR AND PHYLOGENETIC TREE OF THE *BRUCELLA* ISOLATES

T. Dal[1], R. Durmaz[1], A. Ceylan[2], F. Bacalan[3], A. Karagöz[4], B. Çelebi[4], E. Yaşar[5], S. Kılıç[4], C. Açıkgöz[1]

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Zoo-4

PREVALENCE OF BRUCELLOSIS WITH SEROLOGIC METHODS IN BRUCELLOSIS SUSPECTED CASES

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Introduction. Brucellosis is an endemic disease in Turkey. Production of the agent in culture is the gold standard method for the diagnosis of the disease however culture method takes long time like 21 days.

Aim. In this study it was aimed to determine seropositivity rates of Brucella and comparison of diagnostic values of serological methods.

Materials and methods. The sera samples obtained from the Brucella suspected patients that applied to Afyon Kocatepe University ANS Practice and Research Hospital. First Rose Bengal Test was performed. The samples detected as positive with Rose Bengal Test was examined with Brucella tube agglutination test. ELISA method used for determination of Immunglobuline M and G antibodies of Brucella.

Results. A total of 6471 sera samples enrolled in to the study. A thousand and three hundred and seventy of samples was detected as positive by Rose Bengal method. Of the total samples 355 was found 1/160 and over titres positive. ELISA method was preferred rarely in our hospital; 35 of the sera samples was inversigated for IgG and IgM antibodies. Five of samples was determined positive for IgM, six of

Zoo-6

COMPARISON OF DIFFERENT DNA EXTRACTION METHODS FOR DETECTION OF BRUCELLOSIS IN BLOOD CULTURE BOTTLES

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Aims. *Brucella* bacteria are among pathogens with a high risk of transmission to laboratory workers. In order to remove contamination risk in conventional methods, using molecular methods for identification of *Brucella* from positive cultures is important. This study was aimed to compare two kits for Brucella DNA extraction from blood culture bottles.

Materials and methods. A *Brucella melitensis* positive blood culture bottle was gently shaked and 100 µL of this culture was inoculated into 10 different blood culture bottles. Then 10 mL of Brucella-negative whole blood was added to each of the bottles. The inoculated bottles were incubated. DNA extractions were performed from the positive signaling blood culture bottles usin
isolation kit (Kit I) and the Thermo Scientific GeneJe
purification kit (Kit II). Multiplex Real-time PCR (qPC
Quantitec master mix with the primer/probes specifi
and GAPDH.

Zoo-9

DEVELOPMENT OF ELISAs AND EVALUATION OF BRUCELLACAPT AND TWO RAPID SEROLOGICAL TESTS FOR THE DETECTION OF HUMAN BRUCELLOSIS

O. Genç[1], Y. Çetinkol[2], Ö. Büyüktanır Yaş[3], N. Yurdusev[4]

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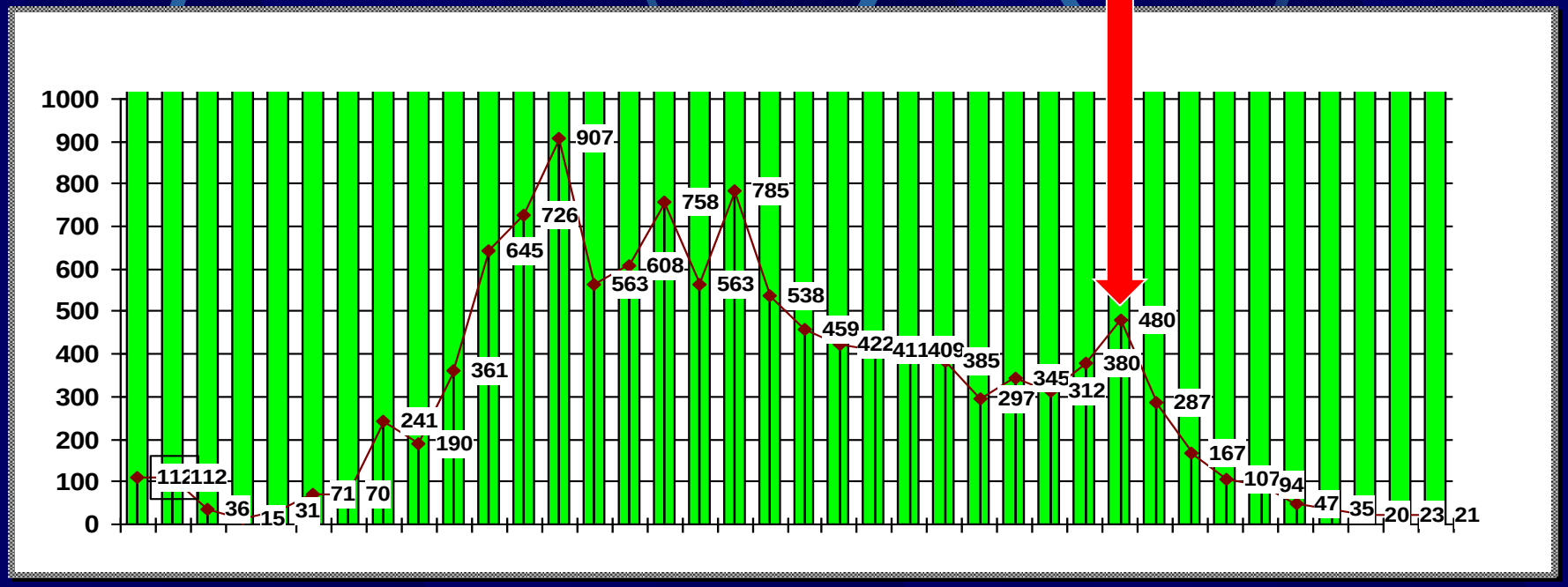
[4]Prof. Dr. BioOptimum Health and Biotech Co., Çankaya/Ankara

Aim. As human Brucellosis has several clinical phases, the accurate diagnosis of the disease have some challenges and the search for reliable diagnosis is going on. Therefore in this study, the aims were to develop in-house ELISA for IgG, IgM and IgA antibody detection and to evaluate rapid immunofiltration based tests designated as enzymatic (ERIFA) and non-enzymatic (NERIFA) as alternative serological tests and also to evaluate test dilution factor for Brucellacapt performance.

Methods. The samples of clinically suspicious individuals were obtained from a retrospective study performed in Clinical Microbiology Department of Kars State Hospital between 2007 and 2009. The samples were tested by RBT and Wright test. In this study, ERIFA and NERIFA tests were performed as reported by Genç et al and the other tests were done as recommended by OIE.

Macedonia:
Area: ~25.000 km²
Population: ~2.000.000



Human *brucellosis* in Macedonia
(1980-2017, ~12.000 human cases)

Brucellosis control strategy in Macedonia

Until 2008, existing system "test and slaughter" was not successful, so high numbers of infected animals and humans (average 400-500 new cases per year) were stable.

In 2008, control strategy was completely changed to vaccination for small ruminants (sheep and goats) with Rev 1 vaccine, applied intraocular.

As a result of vaccination of small ruminants (Rev 1) started in 2008, number of human cases significantly decreased.

YEAR	HUMAN CASES
2008	480
2009	287
2010	167
2011	107
2012	94
2013	47
2014	35
2015	20
2016	23
2017	21

For differentiation of Rev 1 strain from infectious/field strains PCR-RFLP based on a mutation in the rpsL gene was implemented.

УНИВЕРЗИТЕТ „СВ.КИРИЛ И МЕТОДИЈ“ СКОПЈЕ

ФАКУЛТЕТ ЗА ВЕТЕРИНАРНА МЕДИЦИНА

Кирил Крстевски

Molecular typisation and phylogenetic analysis od bacteria of *brucella* detected on the territory of Republic of Macedonia (doctoral dissertation - 2015)

1. Isolation
2. Identification determining IS711 using **rt-PCR**
3. *Brucella* species determined by **AMOS PCR**
4. Genotyping using method **MLVA – 16**

- Genetic similarity and phylogenetic grouping according program package ***Bio-numerics 7***

- Significantly bigger differences of species ***B. melitensis*** (**22 genotypes**), belongs to east-Mediterranian species, most similar as **Turkish species** (?).

- In ***B. abortus*** ***only 2 genotypes were proved (most similar with Portuguese species).***

***Brucella hots*:**

- **Mammals**
  - *marine*
  - *terrestrial*
- **Rodents**

- **Amfibija (frogs)?**

Recently reported *brucellae* from amphibians (worldwide-distributed exotic frogs) are ***genetically highly diverse*** and might represent several new *Brucella* species ***or link*** between free living soil saprophytes and the pathogenic *Brucella.*

Amphibian *brucellae* are capable of causing disease in different frog species ranging from localized manifestations to generalized infections.

Frogs represents new and ecologically significant natural host and reservoir.

The Change of a Medically Important Genus: Worldwide Occurrence of Genetically Diverse Novel *Brucella* Species in Exotic Frogs

- Holger C. Scholz ,
- Kristin Mühldorfer,
- Cathy Shilton,
- Suresh Benedict,
- Adrian M. Whatmore,
- Jochen Blom,
- Tobias Eisenberg

PLOS

2016

- Published: December 30, 2016

AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology

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Isolation of Potentially Novel *Brucella* spp. from Frogs

Tobias Eisenberg[a], Hans-Peter Hamann[a], Ute Kaim[a], Karen Schlez[a], Helga Seeger[a], Nicole Schauerte[b], Falk Melzer[c], Herbert Tomaso[c], Holger C. Scholz[d], Mark S. Koylass[e], Adrian M. Whatmore[e] and Michael Zschöck[a]

Author Affiliations

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Accepted manus
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Appl. Environ. Mi
2012 vol. 78 no.
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PDF

Brucella spp. of amphibians comprise genomically diverse motile strains competent for replication in macrophages and survival in mammalian hosts

- Sascha Al Dahouk, Stephan Köhler, Alessandra Occhialini, María Pilar Jiménez de Bagüés, Jens Andre Hammerl, Tobias Eisenberg, Gilles Vergnaud, Axel Cloeckaert, Michel S. Zygmunt, Adrian M. Whatmore, Falk Melzer, Kevin P. Drees, Jeffrey T. Foster, Alice R. Wattam& Holger C. Scholz

- *Scientific Reports* 7, Article number: 44420 (2017)

African Bullfrog

First publication report (Eisenberg T, Hamann HP, Kaim U, Schlez K, Seeger H, Schauerte N, et al. Isolation of potentially novel *Brucella* spp. from frogs. Appl Environ Microbiol. **2012**; 78:3753–3755.)

Wild-caught African bullfrogs (*Pyxicephalus edulis*) imported from Tanzania in a quarantine centre of a zoo in Germany, isolated from a granulomatous / purulent skin lesion

Big-eyed tree frog (Leptopelis vermiculatus)

The second publication (Fischer D, Lorenz N, Heuser W, Kämpfer P, Scholz HC, Lierz M. Abscesses associated with a *Brucella inopinata*-like bacterium in a big-eyed tree frog (*Leptopelis vermiculatus)*. J Zoo Wildl Med. **2012;** 43:625–628).

Reports the isolation of a *Brucella inopinata*-like strain from subcutaneous abscess material of a big-eyed tree frog (*Leptopelis vermiculatus*) bought from a pet shop in Germany

White's Tree Frog (*Litoria caerulea*)

Third case
was reported from the UK in a White's tree frog (*Litoria caerulea*) with fluid-filled skin lesions.

Isolation of Brucella from a White's tree frog (Litoria caerulea)

- Authors: Adrian M. Whatmore[1], Emma-Jane Dale[1], Emma Stubberfield[1], Jakub Muchowski[1], Mark Koylass[1], Claire Dawson[1], Krishna K. Gopaul[1], Lorraine L. Perrett[1], Matthew Jones[2], Alistair Lawrie[3]
- **First Published Online:** 01 February 2015, JMM Case Reports , 2015 2, doi:

2016

Pacman frog (Ceratophrys ornate)

The most recent case of *Brucella* infection was described in a Pac-Man frog (*Ceratophyrus ornate*) at a veterinary hospital in Texas; USA

Soler-Lloréns PF, Quance CR, Lawhon SD, Stuber TP, Edwards JF, Ficht TA, Robbe-Austerman S, O'Callaghan D, Keriel A. A Brucella spp. Isolate from a Pac-Man Frog (*Ceratophrys ornata*) Reveals Characteristics Departing from Classical Brucellae. Front Cell Infect Microbiol. 2016; 28:116.

Meanwhile ‘atypical’ *brucellae* were also identified in :

Tomato frog (Dyscophus antongilii)

Red-eyed tree frog (Agalychnis callidryas)

Amazonian milk frogs (*Trachycephalus resinifictrix*)

Cane toads (*Chaunus marinus*)

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International Journal of Veterinary Science and Medicine

journal homepage: www.elsevier.com/locate/ijvsm

Brucellosis: Evolution and expected comeback

Amr El-Sayed*, Walid Awad

Faculty of Veterinary Medicine, Department of Medicine and Infectious Diseases, Cairo University, Giza, Egypt

2018

Vet. Sci. **2018**, *5*(1), 28; https://doi.org/10.3390/vetsci5010028

Open Access Communication

Isolation of *Brucella abortus* and *Brucella melitensis* from Seronegative Cows is a Serious Impediment in Brucellosis Control

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2 Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Bacterial Infections and Zoonoses, 07743 Jena, Germany

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CONCLUSIONS (1)

- Control of animal brucellosis (trading, transport and slaughter) control of animal products: meat, milk and their products) is imperative for control of human brucellosis

- Permanent long-term financial support of control programs, institutional and regional cooperation

- Education of the population and professionals

- New trends warn that Brucellosis must not be neglected even in countries which achieved satisfactory control on brucellosis

- Introducing molecular methods for detection and genotyping of *Brucella spp*., may significantly increase efficacy in detection of new reservoirs and epidemiological connection

CONCLUSIONS (2)

New *brucella* strains, new hosts and large reservoirs makes control of Brucellosis more complicated.

Identification of “atypical” *Brucella* strains and new amphibian, *Brucella* species and new hosts and reservoirs, have significant contribution to understanding of evolution of the genus *Brucella* from a soil-associated motile bacterium to a host-adapted pathogen.

To date, there is no evidence that frog’s isolates represent a zoonotic threat, but precaution to avoid contacts with potentially infected amphibians until the zoonotic potential is better investigate and understood is useful advice.


Kiss
Me!

Thank you very much for your attention